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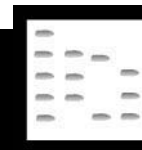
ALCOHOL AND BIOLOGICAL MARKERS OF ALCOHOL ABUSE: GAS CHROMATOGRAPHY



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The use of alcoholic beverages is probably the most ancient social habit worldwide, but alcohol abuse has generated severe problems. Chronic and/or acute alcohol intoxication has been demonstrated to be connected with serious pathologies, suicides, homicides, fatal road and industrial accidents and many criminal offences. Alcoholism is a widespread social, medical and economic problem in a large section of the population of nearly all ethnic groups. Therefore, it is of great importance to have diagnostic tools (biological markers) to detect excessive alcohol consumption and alcoholism. This article deals with gas chromatographic techniques to determine excessive alcohol consumption. The following parameters are described: ethyl alcohol and congeners, ketone bodies, ethyl glucuronide, fatty acid ethyl esters and condensation products like salsolinol.

Ethyl Alcohol

The most obvious and specific test for heavy drinking is the measurement of blood, breath or urine alcohol (ethyl alcohol). However, this simple test cannot distinguish between acute and chronic alcohol consumption, unless it can be related to an increased tolerance of alcohol. According to the American National Council on Alcoholism (NCA), the first-level criteria for the diagnosis of alcoholism are blood alcohol exceeding 1.5 g L^{-1} without gross evidence of intoxication, over 3 g L^{-1} at any time, or over 1 g L^{-1} in routine examination. The determination of alcohol has already been the subject of many reviews. The most important facts are summarized here.

As a first step, various pitfalls and analytical problems such as interference in alcohol analysis induced by cleaning the skin with ethanol or isopropanol before expert venepuncture should be borne in mind. The stability of ethanol during storage is a problem. The main factors affecting alcohol determination in stored blood are the duration and temperature of storage, with negligible losses in the frozen state, and the presence of a preservative. Three mechanisms accounting for these changes are: oxidation (highly

temperature-dependent, needing oxygen from oxyhaemoglobin), the growth of microorganisms metabolizing ethanol (inhibited by sodium fluoride at $\geq 0.5\%$, w/v) and diffusion from containers owing to closure failure. A further potentially interfering factor, especially in autopsy cases, is ethanol production in (postmortem) tissues by bacteria and yeasts. Freezing seems to be the best precaution in order to maintain the original alcohol levels.

Gas chromatography (GC) is *par excellence* the all-purpose technique for the determination of volatile molecules, such as alcohols and related compounds. Almost all GC methods for ethanol determination allow the simultaneous measurement of a wide range of other volatile analytes (alcohols, aldehydes, ketones, glycols, etc.). Although some of the earlier techniques have become obsolete, the incorporation of advances such as headspace chromatography have extended the popularity of chromatography. The analytical conditions of the most interesting methods are summarized in **Tables 1** and **2**. The following classification has been used.

Direct Injection

Methods using direct injection of whole blood suffer from the adsorption of undesirable compounds (proteins and other macromolecules) on the column and, consequently, in most procedures prior dilution or centrifugation have been used.

With Extraction

For a prior extraction step organic solvents such as *n*-propyl acetate, *n*-butanol or dioxan are used.

With Distillation

Sample and internal standard in sodium tungstate/sulfuric acid are subjected to distillation. The distillate is injected into the column and detection is performed by thermal conductivity or flame ionization.

Table 1 Direct injection gas chromatography. Representative overview of standard procedures for the determination of ethyl alcohol

Specimen (mL or g)[a]	*Diluent (mL)*	*Column (m × mm I.D.)*	*Packing (mesh)*	*Oven temperature (°C)*	*Carrier gas (mL min^{-1})*	*Detection*	*Internal standard*
Blood (0.5)	Int. standard solution (0.5)	1.8 × 6	30% Carbowax 20M on Chromosorb W (60–80)	100	Nitrogen (35)	FID	Isobutanol
Blood (0.01)	Int. standard solution (0.1)	1.5 × 4.8	10% Carbowax 400 on Chromosorb W (80–100)	75	Nitrogen (75)	FID	*n*-Propanol
Blood Urine Serum Plasma (0.5 μL)	Int. standard solution (0.5 μL)	2 × 3	(1) 0.2% Carbowax 1500 on Carbopack C (80–100)	120		FID	*n*-Propanol
			(2) 30% Carbowax 20M on Chromosorb W HP (60–80)	100	(20)		Isobutanol
Serum (0.1)	Int. standard solution + Triton-X-100 (0.1)	3 × 3.2	Porapak Q (80–100)	155	Nitrogen (18)	FID	Acetonitrile
Serum (0.2)	Int. standard solution (0.2) Sodium tungstate 0.2 mol L^{-1} (0.2) Copper (II) sulfate 0.2 mol L^{-1} (0.2)	30 × 0.25	Methylsilicone-bonded phase (0.25 μm)	35	Helium	FID	*n*-Propanol
Blood	Water (50-fold sample vol.)	15 × 0.53	Polyethylene glycol (1.0 μm)	40	Helium (25)	FID	
Blood Urine Serum Plasma	Int. standard solution (twofold)	1.8 × 2	Porapak S (80–100)	165	Nitrogen (45)	FID	Acetonitrile
Blood (0.1–0.3)	Sodium tungstate 12.5% (0.2) Sulfuric acid 0.33 mol L^{-1} (0.2)	2 × 3	Porapak Q (80–100)	180	Nitrogen (30)	FID	Isopropanol
Blood (0.2)	Int. standard solution (0.8)	1.2 × 4	5% Carbowax 20M on Supelcoport (100–120)	100	Helium (30)	FID	*n*-Butanol

[a]mL for serum/plasma/urine or g for blood.

Selection according to Tagliaro *et al.* (1992) Chromatographic methods for blood alcohol determination. *Journal of Chromatography* 580: 161.

Table 2 Headspace gas chromatography. Representative overview of standard procedures for determination of ethyl alcohol

Specimen (mL or g)[a]	*Incubation*		*Packing (mesh)*	*Oven temperature (°C)*	*Carrier gas (mL min^{-1})*	*Detection*	*Internal standard*
	Temperature (°C)	*Time (min)*					
Blood (0.02)	60	3	Porapack Q (80–100)	150	Nitrogen (30)	FID	*n*-Propanol
Blood Serum (0.5)	60	30	5% Carbowax 20M on Carbopack B (60–80)	65–110	Nitrogen (30)		*n*-Propanol
Blood (0.2)	60	20	(1) 0.2% Carbowax 1540 on Carbopack C (60–80) (2) 15% Polyethylene glycol on Celite (60–100)	85–100		FID	*tert*-Butanol
Blood (0.2)	20–40	30	0.2% Carbowax 1500 on Carbopack C (80–100)	125	Nitrogen (20)	FID	*n*-Propanol
Blood	20–40	30	Methylsilicone	35–40	Helium (25)	FID	*n*-Propanol
Blood (0.5)	55	12	(1) Methylsilicone (megabore) (2) DB-wax (megabore)	45	Helium (7.5)	FID	*n*-Propanol
Blood Urine (0.1)	40	18	(1) 0.2% Carbowax 1500 on Carbopack C (80–100) (2) 5% Carbowax 20M on Carbopack B (60–80) (3) 15% Carbowax 20M on Chromosorb W	100	Nitrogen (20)	FID	*n*-Propanol
Plasma	25	—	Porapak S (80–100)	165	Nitrogen (45)	FID	

[a]mL for serum/plasma/urine or g for blood.

Selection according to Tagliaro *et al.* (1992) Chromatographic methods for blood alcohol determination. *Journal of Chromatography* 580: 161.

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Headspace Methods

The most important advantage is the prevention of column contamination.

Methods requiring solvent extraction or distillation should be considered obsolete mainly because they are time- and sample-consuming and not susceptible to automation. Direct injection and headspace GC are the only techniques in general use that can be fully and easily automated. The description of direct injection technique is mostly connected with the dilution of the sample (mostly with aqueous solutions containing the internal standard) and with the injection of small volumes. Additional protection from contamination can be obtained with a glass sleeve inserted in the injection port or with a pre-column glass insert filled with a silanized glass wool plug. Triton X-100 has been reported to improve the performance of the direct injection of serum by acting as a protein-dispersing agent. Protein precipitation, which can be carried out in conjunction with the addition of the sample with the internal standard, has been proposed as a simple means of overcoming the problems related to the injection of whole blood. Headspace GC for blood alcohol analysis was the subject of a review in 1975 by Machata who made many contributions to the development of this technique. Chromatograms are shown in **Figure 1**. Headspace analysis prevents any contamination of the column and injector with involatile material and is preferred in routine laboratories. Also, reproducibility is often better than in direct injection (typical within and between-run coefficients of variation $< 1.5\%$ and $< 2.5\%$, respectively). Analytical problems arise concerning the choice of the sample equilibration temperature; oxidation of ethanol takes place at temperatures exceeding 40°C, but higher temperatures increase the air–blood partition coefficient and, consequently, the sensitivity. The conversion of ethanol into acetaldehyde is reportedly inhibited by the addition of sodium nitrite or sodium dithionite. Increased sensitivity due to a salting-out effect is obtained using sodium chloride, sodium nitrite, potassium carbonate, sodium fluoride and ammonium sulfate. In such non-ideal solutions, the vapour pressures of volatile components at a fixed temperature have been reported to depend on the water content of the sample.

An additional advantage of headspace technique is the complete elimination of matrix-related effects, which prompted its use for the analysis of tissues, stool samples or other biological material. A new procedure is the headspace–solid-phase microextraction

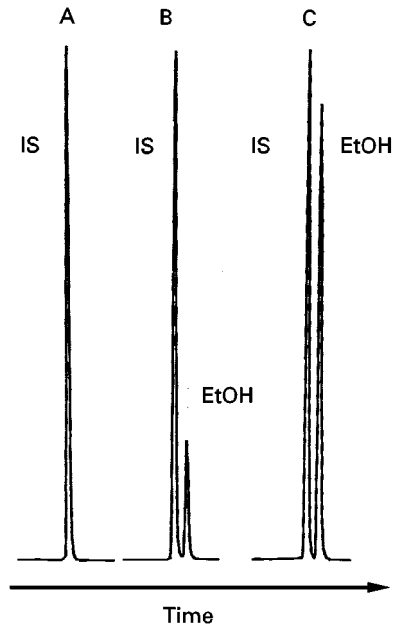

Figure 1 Representative headspace gas chromatograms determining alcohol concentrations in human serum samples. A, Blank (serum); B, 0.48 g L^{-1}; C, 1.95 g L^{-1}. Retention times: EtOH, 1.65 min; t-butanol, 2.2 min.

(HS-SPME) technique, based on the adsorption of analytes directly from the headspace on to a coated fused silica fibre. Various fibres for different analytes are available and a 65 μm Carbowax/divinylbenzene coating is used for alcohols.

Alcohols can be efficiently separated with different GC columns and the choice is often only based on practical considerations such as total analysis time, cost, column life and the possibility of using the same column for different analyses. Carbopack B coated with Carbowax 20M is superior to Carbopack C coated with Carbowax 1500 for the determination of acetaldehyde and methanol and is also superior to adsorption chromatography on Porapak Q and Chromosorb 102. Separation is generally carried out under constant temperature conditions; temperature programming has been used for the simultaneous determination of less volatile compounds. Detection is universally carried out by a flame ionization detector (FID). Capillary chromatography (Carbowax 20M) allows a higher separation performance and easier coupling with mass spectrometry, which is preferred for the determination of lower volatile alcohols.

Congeners

Besides ethanol, alcoholic beverages contain up to 800 flavour compounds and some of these congeners can be found in sufficient quantities to allow their detection in the blood of the consumer. There are characteristic differences in the congener content of alcoholic beverages. A close correlation between the consumed amount of a congener alcohol and the resulting blood level can be helpful for the evaluation of allegations concerning alcohol intake in forensic cases, especially when determining types of drinks and when estimating the time of drinking (**Figure 2**). The sensitivity of conventional headspace GC is sufficient for blood ethanol determinations down to 0.01 g L^{-1}, but for the detection of congener alcohols the limits of detection had to be improved to 0.01 mg L^{-1}. Some procedures contain special sample preparation steps, which include homogenization by ultrasound and/or ultrafiltration. As the long chain alcohols are partly or completely bound to glucuronic acid, incubation with β-glucuronidase is necessary. Using a temperature programme and capillary columns the loading capacity can be enhanced by a cryofocusing technique.

Methanol is an important congener of most alcoholic beverages. Metabolism of methanol via liver alcohol dehydrogenase is inhibited by ethanol levels exceeding 0.4 g L^{-1}. Consequently, excessive and prolonged drinking results in high blood methanol levels. Increased blood methanol levels are frequently found in drunken drivers and alcoholics. On the basis of these findings, blood methanol levels exceeding 10 mg L^{-1} have been suggested to be an indicator of alcoholism. Additionally higher concentrations of acetone and propanol-2 have been proposed as an indication of drinking behaviour. This phenomenon is caused by reciprocal formation through the alcohol dehydrogenase system. If the sum of the concentrations exceeds 9 mg L^{-1}, heavy drinking is suspected. However, due to the effects of metabolic disorders (ketosis, diabetes, hunger, physical stress), the significance has been regarded as very low.

Ketone Bodies

In many forensic cases alcohol abusers have been found dead and the cause of death cannot be ascertained. In order to examine the possible role of ketoacidosis as the cause of death the concentrations of ketone bodies (acetone, acetoacetate, D-β-hydroxybutyrate) have to be determined in postmortem blood specimens. The phenomenon of ketoacidosis is often seen as typical in periods of abstinence with low intake of food. It is due to the accumulation of D-β-hydroxybutyrate and acetoacetic acid. The accumulation is probably the result of various factors such as volume depletion and starvation, which have a lipolytic effect.

A routine procedure is a coupled enzymatic headspace GC method (**Figure 3**). This procedure is based

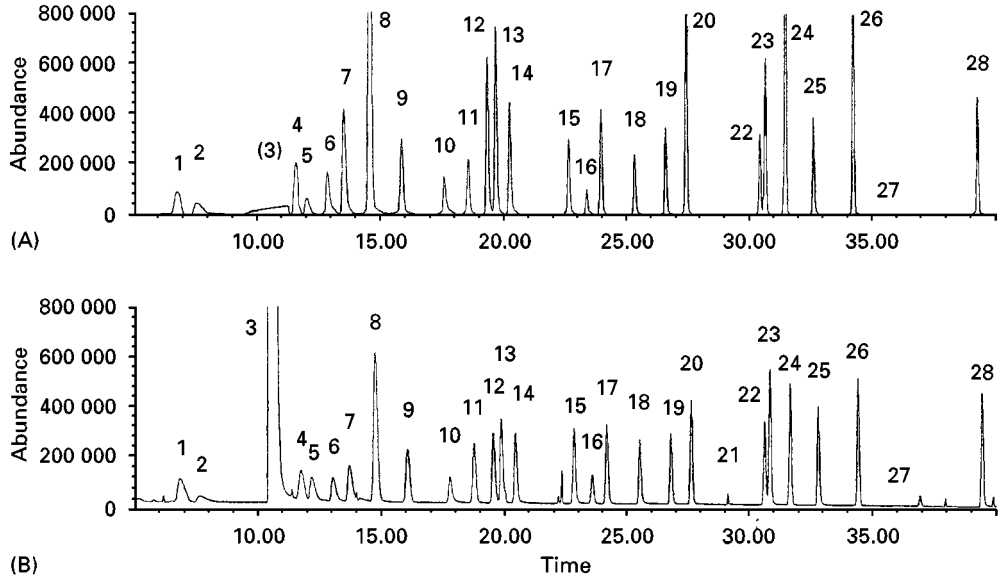

Figure 2 Total ion chromatograms ((A) selected ion monitoring and (B) full scan mode) of a standard solution of 28 substances relevant in congener analysis in concentrations of 2 mg L^{-1} (methanol 10 mg L^{-1}, acetaldehyde 0.5 mg L^{-1}). 1, Acetaldehyde; 2, methanol; 3, ethanol; 4, propionaldehyde; 5, acetone; 6, propanol-2; 7, methyl acetate; 8, *t*-butanol (internal standard); 9, *i*-butyraldehyde; 10, propanol-1; 11, *n*-butyraldehyde; 12, methyl ethyl ketone; 13, ethyl acetate; 14, butanol-2; 15, *i*-butanol; 16, *i*-valeraldehyde; 17, 2-methylbutyraldehyde; 18, butanol-1; 19, *n*-valeraldehyde; 20, 1,1-diethoxyethane; 21, 3-hydroxybutanone-2; 22, 3-methylbutanol-1; 23, 2-methylbutanol-1; 24, *i*-butyl acetate; 25, pentanol-1; 26, butyl acetate; 27, ethyl lactate; 28, hexanol-1. GC parameter: HP 5890 II GC with HP MSD 5972, equipped with a DB 624 column (60 m × 0.32 mm, df = 1.8 μm); helium flow 1 mL min^{-1}; injector 150°C; detector 200°C; oven initially 30°C for 8 min, 3°C min^{-1} up to 180°C. (Reproduced with permission from Roemhild W (1998) Congener analysis by means of 'headspace'– GC/MS. *Blutalkohol* **35**: 10.)

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on enzymatic dehydrogenation of D-β-hydroxybutyrate into acetoacetate and subsequent decarboxylation of this compound into acetone. Three portions are taken from each sample. One portion is heated to 60°C in a headspace sampler, which gives the free acetone. Acetoacetate is converted into

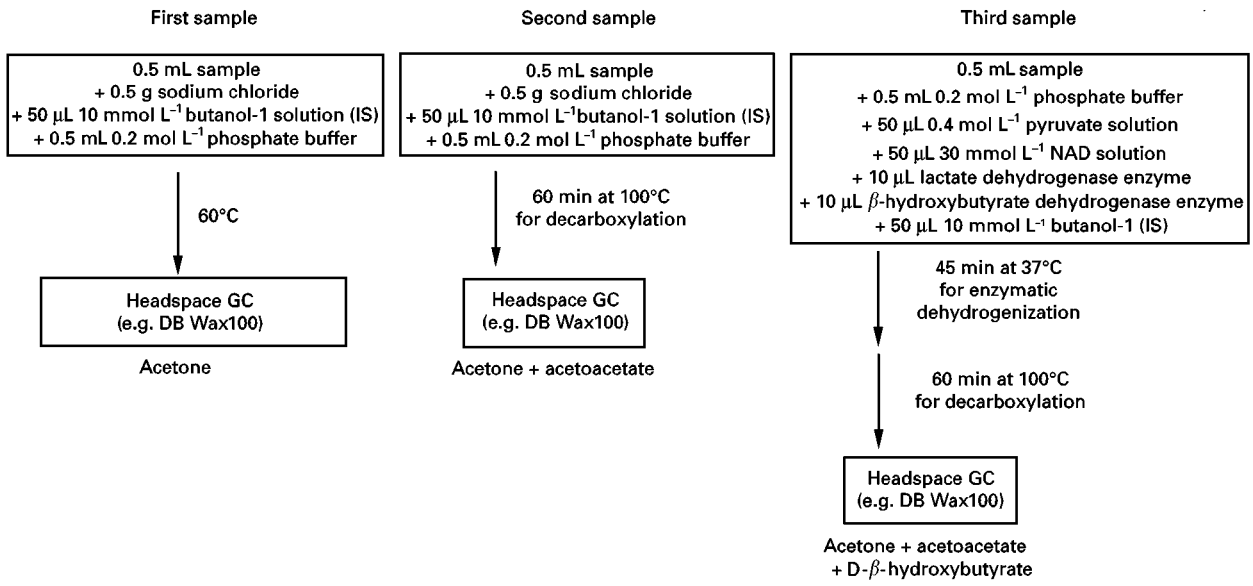
First sample
0.5 mL sample
+ 0.5 g sodium chloride
+ 50 μL 10 mmol L-1 butanol-1 solution (IS)
+ 0.5 mL 0.2 mol L-1 phosphate buffer
60°C
Headspace GC
(e.g. DB Wax100)
Acetone
Second sample
0.5 mL sample
+ 0.5 g sodium chloride
+ 50 μL 10 mmol L-1 butanol-1 solution (IS)
+ 0.5 mL 0.2 mol L-1 phosphate buffer
60 min at 100°C
for decarboxylation
Headspace GC
(e.g. DB Wax100)
Acetone + acetoacetate
Third sample
0.5 mL sample
+ 0.5 mL 0.2 mol L-1 phosphate buffer
+ 50 μL 0.4 mol L-1 pyruvate solution
+ 50 μL 30 mmol L-1 NAD solution
+ 10 μL lactate dehydrogenase enzyme
+ 10 μL β-hydroxybutyrate dehydrogenase enzyme
+ 50 μL 10 mmol L-1 butanol-1 (IS)
45 min at 37°C
for enzymatic
dehydrogenization
60 min at 100°C
for decarboxylation
Headspace GC
(e.g. DB Wax100)
Acetone + acetoacetate
+ D-β-hydroxybutyrate

Figure 3 Schematic presentation of a standard procedure for determination of ketone bodies in blood specimens. Three portions are taken from each sample to determine free acetone and the sums of acetone + acetoacetate and acetone + acetoacetate + D-β-hydroxybutyrate.

acetone by decarboxylation at 100°C in the second portion. This part gives the combined amount of acetone and acetoacetate. In the third portion, D-β-hydroxybutyrate is first enzymatically dehydrogenized into acetoacetate by D-β-hydroxybutyrate dehydrogenase and then decarboxylated into acetone. Quantification of acetone then yields the molar equivalent of the total ketone bodies. Omission of the enzymatic stage of the analysis allows quantification of the molar equivalent of acetone and acetoacetate present, and the substraction of this value from total ketone quantitation allows calculation of the D-β-hydroxybutyrate concentration.

The reported ketone body concentrations vary a lot. It was held that if the ketone body concentration of the blood exceeds 531 μmol L^{-1} and if there is no other plausible cause of death in a group of alcohol abusers, the term ketoalcoholic death should be used. In another study it was pointed out that very hight levels, above 10 mmol L^{-1}, are indicative of profound alcoholic ketoacidosis.

Ethyl Glucuronide

Ethyl glucuronide (EtG) is a minor metabolite of ethanol and is formed from ethanol by conjugation with uridine diphosphate (UDP)-glucuronic acid. EtG has been detected in human urine, serum and clipped hair samples of ethanol consumers. The formation of EtG depends on the serum ethanol concentration. It was shown that serum EtG concentrations higher than 5 mg L^{-1} may indicate alcohol misuse, especially if the serum ethanol concentration is less than 1 g L^{-1}. The EtG concentration declines exponentially with a half-life of 2–3 h and testing for EtG is restricted to a period of about 6–18 h after drinking, depending on the ethanol dose and individual metabolism. In forensic cases testing is predominantly indicated when the ethanol determination gives negative results and consumption is denied. For retrospective studies detection of EtG in hair samples also seems to be possible. However, if excessive ethanol consumption over a period of months or years provokes a stimulation of glucuronyltransferase in the liver, the extent of the EtG formation might be an indicator of ethanol abuse.

For the determination of EtG in serum the sample is precipitated with acetone or methanol and the dried supernatant is derivatized by addition of acetic anhydride and pyridine. A mass spectrum of the triacetyl derivative is shown in **Figure 4**. Hair samples are extracted with methanol, including treatment by ultrasound prior to derivatization. On an OV-1 capillary column, the retention index is 1920. Gas chromatography–mass spectrometry (GC-MS) was performed with an electron energy of 70 eV and gave the following m/z values (intensities higher than 20% in parentheses): 85 (53), 88 (41), 101 (38), 113 (66), 114 (42), 115 (100), 117 (47), 130 (25), 157 (73), 142 (25) and 143 (28); there is no parent peak. An m/z value of 303 (1%, M-45) indicates that EtG is decarboxylated.

Fatty Acid Ethyl Esters

Fatty acid ethyl esters (FAEE) are formed by an enzyme-mediate esterification of ethanol with fatty acids or fatty acyl-coenzyme A. It has been shown that FAEE and the FAEE synthase are predominantly present in those organs most often damaged by ethanol abuse, notably the pancreas and liver. This has led to speculation that FAEE, lipids more hydrophobic than triglycerides, are mediators of ethanol-induced organ damage. Following ethanol consumption by humans, FAEE have also been found in serum lipoproteins. Recently it was reported that the concentration of FAEE in the blood closely parallels the concentration of blood ethanol. In serum samples of

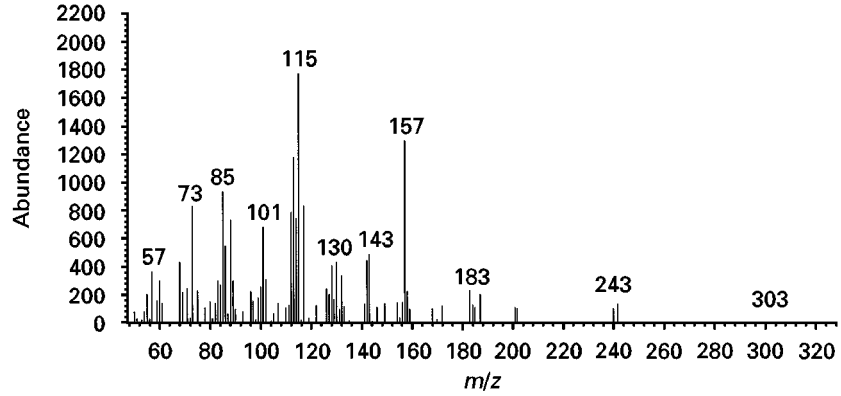

Figure 4 Mass spectrum of the triacetyl derivative of ethyl glucuronide.

subjects who had blood ethanol concentrations $> 1.5\ g\ L^{-1}$, FAEE concentrations ranged up to $2500\ nmol\ L^{-1}$ and were still detectable 24 h after ethanol ingestion. However, serum FAEE may evolve into both a short-term and long-term marker of ethanol ingestion. In forensic cases the determination of a recent intake of ethanol may be necessary. A negative blood ethanol with a positive FAEE test is consistent with ethanol intake 4–24 h before blood collection. Additionally it has been reported that FAEE are present in significantly higher amounts in postmortem adipose tissues obtained from individuals with a history of chronic alcohol abuse, with ethanol-induced organ damage at autopsy and zero blood ethanol at the time of death (mean $\pm$ SEM equals $300 \pm 46\ nmol\ g^{-1}$) compared to those from a control group without a history of chronic ethanol ingestion, without ethanol-related organ damage and with zero blood ethanol at the time of death ($43 \pm 13\ nmol\ g^{-1}$; **Figure 5**).

Studies on FAEE frequently involve isolating the compounds by liquid–liquid extraction and thin-layer chromatography (TLC) prior to identification and quantification. The isolation of FAEE by these methods is especially suitable for adipose tissue. Sample material (1–2 g) is extracted in acetone (10% w/v) and the lipids are separated by TLC on silica gel using a petroleum ether/diethyl ether/acetic acid (75:5:1) solvent system. Fatty acid ethyl esters, $R_F = 0.5$, are identified by comparison with standards and eluted from the silica gel with acetone. The reproducibility of this procedure is sometimes a problem and the method often results in low yields. The small amounts of the very hydrophobic FAEE present in human plasma after ethanol ingestion are commonly lost during extraction. As with fatty acids, FAEE moieties which contain two or more double bonds can be oxidized within minutes on a dried TLC plate and are thereby lost prior to quantification. To enhance the recovery of the relatively small amounts of FAEE, an effective solid-phase extraction (SPE) method for isolation is preferred. Extraction of FAEE from serum is initiated by the addition of acetone/hexane solution. After being dried and resuspended in hexane the extract is applied to an aminopropyl silica SPE column with simultaneous elution of FAEE and cholesteryl esters from the column with hexane. The FAEE can then be separated from cholesteryl esters, if necessary, by chromatography on an octadecylsilyl (ODS) SPE column and elution with isopropanol/water (5:1, v/v). Recently a relationship between various levels of alcohol consumption and the appearance of fatty acid methyl esters (FAME) in postmortem tissue samples have been reported. In addition, this connection is supposedly caused by the accumulation of the congener alcohol, methanol, during chronic alcohol abuse.

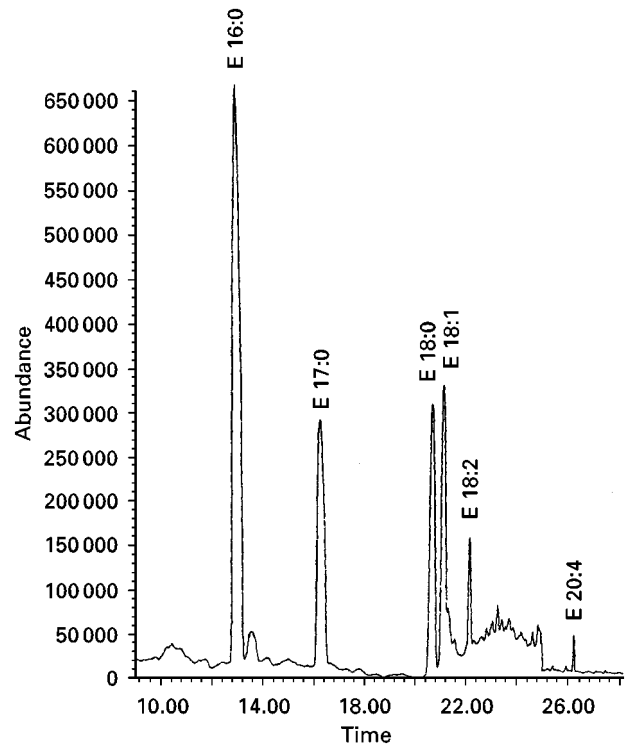

Figure 5 Analysis of FAEE from human plasma. Lipids from sera of patients with markedly elevated blood ethanol levels were extracted into hexane and applied to an aminopropyl silica column. Lipids eluted from the column were dried under nitrogen to a small volume and an aliquot injected into a gas chromatograph – mass spectrometer (WCOT Supelcowax capillary column). The peaks identified as FAEEs are labelled: E 16:0, ethyl palmitate; E 17:0, ethyl heptadecanoate; E 18:0, ethyl stearate; E 18:1, ethyl oleate; E 18:2, ethyl linoleate; E 20:4, ethyl arachidonate. (Reproduced from Bernhardt TG *et al.* (1995) Purification of fatty acid ethyl esters by solid-phase extraction and high-performance liquid chromatography. *Journal of Chromatography B* 675: 189, with permission from Elsevier Science.)

The GC analysis of FAME after esterification of lipids was the subject of an excellent review by Eder in 1995 and the comments are applicable to FAEE. The most critical step in the GC analysis of FAME is sample introduction. The classical split injection technique, which is the most widely used procedure, has the potential disadvantage of boiling-point discrimination. Cold injection of the sample, either on-column or by programmed-temperature vaporization, does not present this problem and is therefore preferred. Separation of FAME can be carried out with nonpolar, polar and very polar stationary phases. The polarity influences the retention times, especially those of polyunsaturated FAME. The resolution capability is highest in columns with very polar phases. However, very polar phases have a shorter lifetime

Table 3 Selection of procedures for determination of tetrahydroisoquinolines (TIQ) and tetrahydro-β-carbolines (THBC)

Sample material	*Analytes*	*Work-up procedure*	*Packing (mesh)/column (m × mm I.D.)*	*Limit of detection*
Tissue and body fluids	Various TIQs and catecholamines	Al_2O_3 extraction; fluoracylation; GC with electrochemical detection (GC-ECD)	3–5% OV-17, SE-30, SE-54, XE-60 or GE XF-1150 on Gas Chrom Q (80–100) (6 ft × 2)	0.2–50 pg per sample
Brain	Salsolinol	Liquid–liquid reextraction; fluoracylation; GC-ECD	3% OV-1 on Gas Chrom Q (100–120) (6 or 8 ft × 2)	10 pg per sample
Urine	TIQs	Liquid–liquid reextraction; trimethylsilyl (TMS) derivatives; GC with mass spectrometry (MS)	3% OV-1 on Gas Chrom Q (100–120) (6 ft × 2)	
Blood, platelets, plasma and brain	Various THBCs	Liquid–liquid reextraction; heptafluorobuturyl (HBF) derivatives; GC-MS	2% SP-2250 (4 ft × 2) or SE-30 (15 × 0.3) on Chromosorb W-HP (100–120)	1 pmol per sample
Brain and biological fluids	Salsolinol and others	Al_2O_3 extraction with deuterated standards; fluoracylation; GC-MS	1% OV-17 (2.5 × 2) or SE-54 (25 × 0.2)	1 pmol per sample
Biological fluids and foods	THBCs	Liquid–liquid extraction with deuterated standards; fluoracylation; GC-MS	SE 52 W COT (20 × 0.25)	0.3 pmol mL^{-1}
Brain	Nor salsolinol	Amberlite extraction; propionyl derivatives; GC-MS	2% SP–2250 on Chromosorb W-HP (100–120) (4 ft × 2)	1 ng g^{-1}
Brain and foods	TIQ and *N*-methyl-TIQ	Liquid–liquid extraction; HFB derivatives; GC-MS	3% OV-17 on Shimalite (80–100) (2 × 2.5) or OV-1 or OV-101 or DB-17 (25 × 0.2 mm)	0.25 ng per sample
Brain and foods	Various THBCs	Liquid–liquid extraction; pentafluorobenzyl (PFP) derivatives; GC-MS	SE 52 WCOT (20 × 0.35 mm)	0.1–0.5 ng per sample
Brain and foods	1-methyl-THBC	Liquid–liquid extraction; TFA derivative; GC-MS with negative CI	OV-1701 (25 × 0.25)	10 fg per sample
Urine	Various THBCs and TIQs	Combined liquid–liquid and solid-phase extraction; carbomethoxy/propionyl derivatives; GC-MS	OV-1 (12 × 0.2 mm)	100 pg mL^{-1}
Urine	1-methyl-THBC	Liquid–liquid extraction; derivatization with (*R*)-(−)-2-phenylbutyryl chloride (PBC)-enantiomeric composition; GC-NICI-MS	RTX-cross-bonded SE-30 (30 × 0.25 mm)	
Plasma and urine	Salsolinol and others	Solid-phase extraction over phenylboronic acid (PBA) cartridges; two-step derivatization (TMS-PBC)-enantiomeric composition; GC-MS	BGB-silaren (30 × 0.32 mm)	100 pg mL^{-1}
Urine	Salsolinol	Extraction and derivatization in one step by Schotten–Baumann two-phase reaction utilizing pentafluorbenzoyl-chloride	DB-5 (30 × 0.25)	10 fmol mL^{-1}
Brain	THBC and 1-methyl-THBC	Liquid–liquid extraction; TFA derivatives; GC-NCI-MS	RTX-cross-bonded SE-30 (30 × 0.25 mm)	20 pg per sample
Urine	Salsolinol and norsalsinol	Solid-phase extraction (PBA); propionyl derivative; GC-MS	OV-1 (12 × 0.2 mm)	100 pg per sample

TFA, trifluoroacetyl; CI, chemical ionization; NCI, negative chemical ionization; TMS, trimethylsilyl.

than nonpolar phases and, in many cases, nonpolar phases provide adequate separation. The most important very polar phases are composed of 100% cyanoethylsilicone oil (SP-2340, OV-275), 100% cyanopropylsilicone (CP-Sil 88) or 68% biscyanopropyl/32% dimethylsiloxane (SP-2330).

The most important stationary phases of intermediate polarity are polyethylene glycol (DB-Wax, Supelcowax 10, Carbowax 20M), acidified polyethylene glycol (FFAP), 86% dimethyl/14% cyanopropylphenylpolysiloxane (DB-1701), and methylsilicone polymer, 25% cyanopropyl/25% phenyl/50% methyl (OV-225, DB-225, SP-2300). Intermediate-polarity columns allow acceptable separation of FAME from biological samples such as plasma or adipose tissue and combine the advantages of a relatively high resolution capability with those of a relatively high thermal stability. The most important nonpolar stationary phases are based on methylsilicones (SPB-1, SPB-5), 95% dimethyl/5% diphenylpolysiloxane (DB-5, CP-Sil 8CB) or 100% dimethylpolysiloxane (DB-1, Rt-1, SP-2100, OV-1, OV-101, CP-Sil 5CB). FAEE are eluted according to their boiling points. Therefore, unsaturated compounds elute before being saturated. This elution order is the reverse of that on very polar and polar columns. The main disadvantage of nonpolar columns is partial overlapping of some unsaturated FAME. Advantages are high thermal stability, a wide range of operating temperatures and chemical inertness.

In summary, FAEE detection can lead to a major improvement in the monitoring of ethanol ingestion and the treatment of ethanol-induced organ damage.

Condensation Products

During the past decades research in the aetiology of alcoholism has focused on the hypothesis that condensation products formed endogenously by the reaction of indolalkylamines and catecholamines with aldehydes or pyruvic acid might be implicated in neurochemical mechanisms underlying addictive alcohol drinking. The formation of 1,2,3,4-tetrahydro-β-carbolines (THBC) and 1,2,3,4-tetrahydroisoquinolines (TIQ) via the Pictet–Spengler reaction is

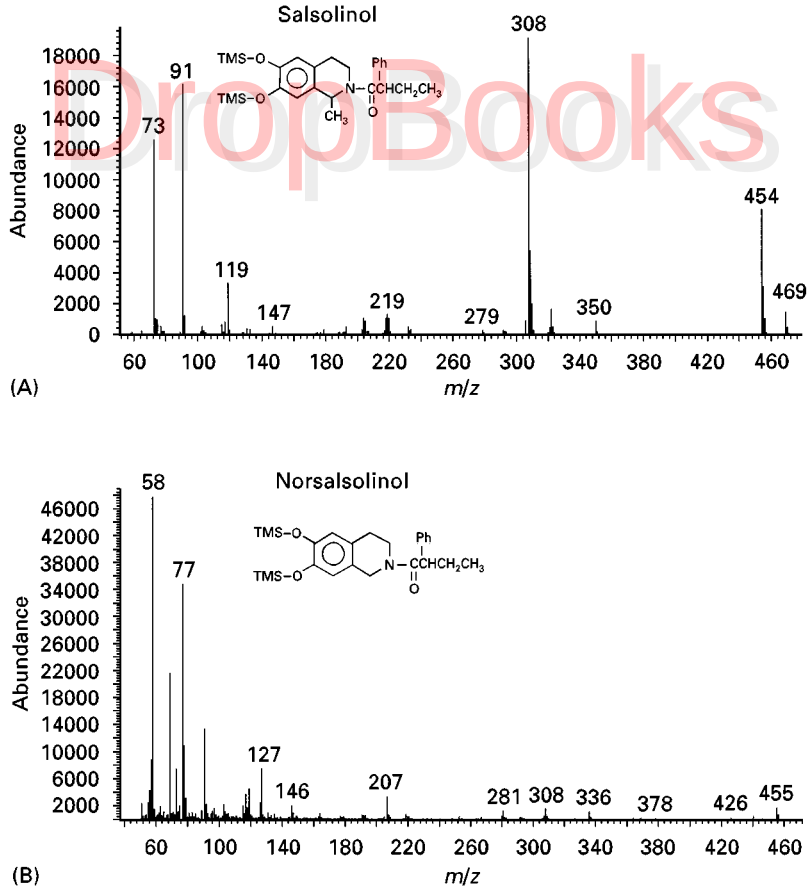

Figure 6 Electron impact mass spectra of (A) salsolinol and (B) norsalsolinol after derivatization with N-methyl-N-trimethylsilyltrifluoracetamide (MSTFA) and (*R*)-(−)-2-phenylbutyrylchloride.

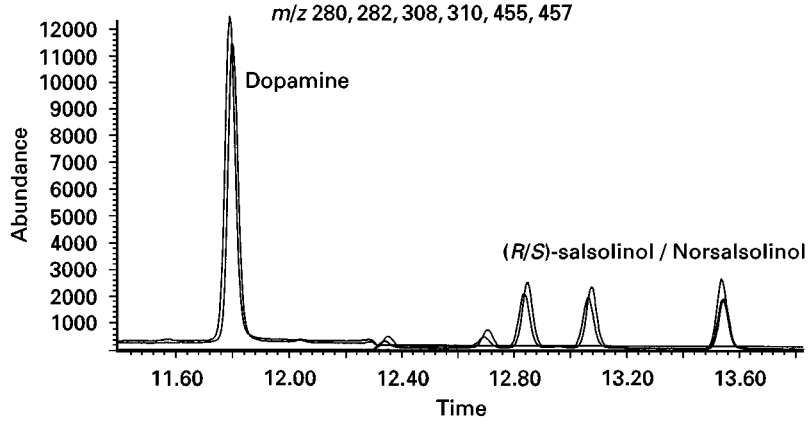

Figure 7 Identification of dopamine, (*R*)-(+)- and (*S*)-(−)-salsolinol and norsalsolinol in an authentic urine sample of a chronic alcoholic.

extensively documented. Salsolinol, which might be formed *in vivo* by ring cyclization of dopamine with acetaldehyde, is one of the most discussed tetrahydroisoquinolines. Several studies have been done to improve analytical techniques for identification in human urine, plasma, brain and cerebrospinal fluid samples. Poor assay specificity and possible artefact formation of the alkaloids during work-up and storage have been suggested to be responsible for controversial reports on the detection of these compounds in mammalian tissues and fluids after alcohol intake. The variability of reported levels of Salsolinol might also be a result of variables, including dietary conditions during the experiments or the duration of ethanol ingestion and analytical problems associated with the detectability of the analytes. The presence of TIQ and THBC compounds has been established using (radioenzymatic) TLC methods, high performance liquid chromatography coupled with electrochemical or fluorescence detection, or GC procedures mostly combined with mass spectrometry (**Table 3**). Recently, it has been considered that the (*R*)-(+)- and (*S*)-(−)- enantiomers of salsolinol do not exert identical biological activities. Thus, methods for the determination of the enantiomeric composition of endogenous salsolinol have been developed (**Figures 6** and **7**). More experimental work is necessary to determine whether alcohol really has an influence on the biosynthesis of salsolinol or other condensation products and if the (*S*)-(−)-salsolinol enantiomer is a sufficient clinical marker to distinguish between alcoholics and nonalcoholics.

Conclusion

Several chemical abnormalities associated with excessive alcohol consumption are useful in the diagnosis of alcoholism. Additionally, in forensic cases information can be helpful to evaluate allegations concerning alcohol intake, especially when determining the types of drinks and estimating the time of drinking. In these problems GC procedures measuring the concentration of ethyl alcohol and congeners or EtG can be helpful. The determination of ketone bodies is a diagnostic tool in a prospective postmortem toxicology analysis in alcoholics for considering a ketoalcoholic death. Further studies are necessary to determine the connection between alcohol abuse and the formation of FAEE and condensation products. Further investigations could lead to important pathopysiological bases of alcohol drinking behaviour and ethanol-induced organ damage and ultimately to better forms of prevention and therapy.

See also: **II/Chromatography: Gas:** Headspace Gas Chromatography. **Detectors:** Mass Spectrometry. **III/Clinical Diagnosis:** Chromatography. **Forensic Sciences:** Liquid Chromatography.

Further Reading

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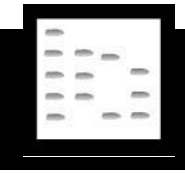
ALCOHOLIC BEVERAGES: DISTILLATION

See **III / WHISKY: DISTILLATION**

ALDEHYDES AND KETONES: GAS CHROMATOGRAPHY



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Introduction

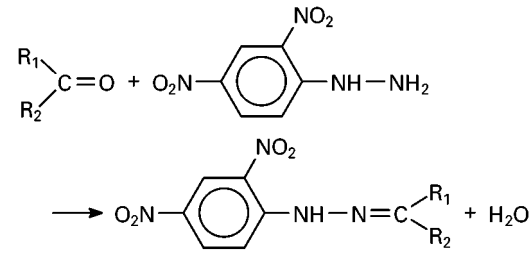
Simple aldehydes, such as formaldehyde, acetaldehyde and acrolein, are known to be hazardous air pollutants. Aldehydes are emitted from incomplete burning of various organic compounds and from various chemicals, and are formed by photochemical reaction with hydrocarbons in the atmosphere.

Volatile ketones are used as solvents in various chemical plants and laboratories and are emitted into the atmosphere. The toxicity of ketones is, in general, not as high as that of aldehydes. Carbonyl compounds are significant in environmental chemistry, i.e. in rainwater and as a photochemical oxidant.

Separation of aldehydes and ketones is very important for the determination of volatile aldehydes. Usually, analysis of aldehydes is performed by derivatization and gas chromatography (GC) or high performance liquid chromatography (HPLC). Selective and sensitive gas chromatographic methods for separation of aldehydes and ketones are described below.

2,4-Dinitrophenylhydrazone Derivatization

2,4-Dinitrophenylhydrazone (DNPH) derivatives of aldehydes and ketones have been used in gas chromatography for many years. The reaction procedure of aldehyde or ketone is as follows:



Kallio *et al.* analysed 15 carbonyl compounds (aldehydes and ketones) known to be flavour components by derivatization/GC with DNPH. The DNPHs of the carbonyl compounds were prepared by shaking 100 μL of each compound with 100 mL of a saturated solution of DNPH in aqueous 2 mol L^{-1} hydrochloric acid and allowing the mixture to stand at room temperature overnight. The precipitated DNPHs were dissolved in ethyl acetate, then analysed by GC-FID or dissolved in benzene and analysed by GC-ECD (electron-capture detector). Packed columns with silicone stationary phases were used. Relative retention times of DNPHs of aldehydes and ketones on one of these columns are listed in **Table 1**